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FACTORS AFFECTING GERMINATION, GROWTH, AND DISTRIBUTION OF THE FRESHWATER SPONGE, *SPONGILLA FRAGILIS* LEIDY (PORIFERA)

THOMAS A. STREKAL AND WAYNE F. McDIFFETT

Department of Biology, Bucknell University, Lewisburg, Pennsylvania 17837

Various field surveys have been conducted to determine which physicochemical parameters affect the distribution of freshwater sponges (Old, 1932; Jewell, 1935, 1939; Cheatum and Harris, 1953; Penney, 1954). Old (1932) concluded that perhaps sponge distribution is influenced by the interaction of environmental factors rather than a single factor, while Jewell (1939) emphasized the role of calcium. Wurtz (1950) summarized the range of values for various parameters affecting individual species distribution. The general biology of the freshwater sponges has been reviewed by Hyman (1940) and Pennak (1953), although there have been few ecological studies dealing with them. No annual survey of a system has been attempted although seasonal fluctuations of important parameters may affect species distribution.

More recent investigations have been devoted to rearing freshwater sponges from gemmules in the laboratory (Rasmont, 1961) to elucidate the physiology of gemmulation and germination, and, more generally, the process of dormancy (Rozenfeld, 1970, 1971; Rasmont, 1962, 1963, 1970; Rasmont and Schmidt, 1967).

This study was initiated to determine (1) the effects of physicochemical factors on sponge gemmule germination and early sponge growth in the laboratory, and (2) which of these factors may be limiting during the year to affect species distribution.

Materials and Methods

The species studied

The freshwater sponge *Spongilla fragilis* inhabits a narrow range in Rapid Run, a mountain stream within R. B. Winter State Park, Union County, Pennsylvania. It is found immediately behind Halfway Dam and in the pool below it attached to smooth concrete surfaces, on submerged twigs, within moss mats, and on the undersides of rocks. It occupies the facing of large rocks at the end of the spillway below the pool and it may be found beyond the spillway for 0.6 mile on the undersides of rocks within the moderately flowing stream. It is not found in the remaining 9 mile course, nor in the stream anywhere above the dam.

Exposed sponges tend to be gray in color; although green sponges (indicating the presence of symbiotic algae) have been reported, none were found during 1972. A white color is characteristic of sponges found under stones.

Germination experiments

Gemmules of *S. fragilis* were collected below the spillway in April, 1972 (one month before germination) by scraping brown mats of them from the undersides of

rocks. These were transferred to a gallon jar filled with distilled water and refrigerated at 2° C until utilized. Identification was made from permanent slides of sponge and gemmule spicules prepared by nitric acid digestion (Pennak, 1953).

Prior to investigation the gemmules were sterilized in a dilute hydrogen peroxide wash and rinsed in distilled water (Rasmont, 1961). Intact gemmules in groups of one, two, and three were selected to facilitate germination and size determinations. These were transferred with surgical forceps to 60 × 15 mm plastic petri dishes containing the various test solutions. Inspection was made to ascertain that no physical damage occurred during transfer.

A threshold temperature for germination was determined by conducting experiments in the dark using three temperatures: 6 ± 1.0° C, 8 ± 0.5° C, and 10 ± 0.5° C. Unless indicated otherwise, germination experiments were conducted at a constant 10 ± 0.5° C, representing an approximate summer mean for Rapid Run. An appliance timer regulated a 60-watt incandescent bulb for a 12-hour-light : 12-hour-dark cycle; dishes were covered with foil for the dark condition.

Measurements were made after 17 days of incubation. Germination was determined by noting the presence of cellular material at the micropyle. Size of the germinated sponge was measured with a Whipple Disc. General morphology was noted in all cases, since the mere presence of cellular material gives no indication of differentiation.

An artificial medium similar in major ion concentration to Rapid Run water was devised to insure consistent conditions in all experiments. Anions and cations were chosen according to Rasmont (1961). Cation concentrations were: magnesium ($MgSO_4 \cdot 7H_2O$) and calcium ($CaCO_3$) — 2 mg/l; sodium ($Na_2SiO_3 \cdot 9H_2O$) and potassium (KCl) — 0.5 mg/l. Suitability of Rapid Run water, Rapid Run Medium (RR Med), and double-distilled water (× 2) as growth media was tested under light-dark and dark conditions in 18 ml of solution. Dilutions were made of RR Med to establish minimum concentration requirements for normal growth, compared to RR Med. The importance of the individual compounds of RR Med was tested by omitting each from the medium, and also by adding each in normal concentration to distilled water; these were grown under 12 : 12, light-dark, conditions. The importance of magnesium, sulfate, calcium, and carbonate was tested by omitting each in turn from RR Med (replacing cations with K^+, and anions with Cl^-). Cations combined with Cl^- and anions with K^+ were also added to distilled water and tested.

Experimental design was as follows: for determining the effect of light and growth media on germination, 5 dishes with 50 gemmules each were used; 4 dishes with 50 gemmules each were used in all remaining experiments.

Statistical methods

Data for per cent germination and size were analyzed using a standard one-way ANOVA table and compared for Least Significance Difference at the 5% and 1% levels.

Field study

A study of a number of environmental parameters (Jewell, 1935) was conducted from March, 1972, to March, 1973, to determine which of them may influence (or

limit) distribution, and to see what effect the dam and lake may have on water quality and hence distribution of sponges.

Sample sites were chosen according to sponge distribution: #1-Rapid Run incurrent to Halfway Dam; #2-immediately downstream from the spillway; #3-approximately 3.7 miles downstream from the spillway.

Temperature and pH were measured on location using a standard mercury thermometer and portable pH meter, respectively. Water samples were taken in one liter polyethylene bottles. All other parameters except silica were tested within one hour in the laboratory. Dissolved oxygen was not determined since turbulence would probably prevent it from being limiting.

Alkalinity was tested by a potentiometric method for low alkalinity (American Public Health Association, 1971). Calcium was measured titrimetrically (EDTA Method, American Public Health Association, 1971). Filtered and unfiltered samples gave identical values for silica, measured colorimetrically (Reactive Silicate, Golterman, 1969). Ionic concentration was determined with a Hach conductivity meter. Apparent color was measured with a Hach portable colorimeter using a #5543 filter.

Sampling was postponed at least two days following rain to circumvent dilution effects.

Results

Gemmules used in the experiments had a mean diameter of 350 $\mu \pm 61$ μ. No correlation was noted between size and the ability of the gemmule to germinate and grow under various light conditions in similar media.

The results of experiments comparing growth media and light versus light-dark conditions are summarized in Figure 1. Sizes were not compared in experiments conducted under dark conditions because fusion of individual sponges had decreased the sample number.

RR Med appeared to be comparable to stream water with regard to germination and growth, regardless of light condition. The two media also produced morphologically similar organisms complete with oscula, canals, and spicule skeletons. Double-distilled water ($\times 2$) produced significantly fewer (1% level) sponges in L:D which were considerably smaller and appeared undifferentiated. Germination in distilled water in total dark was significantly less (1% level) than L:D.

The experiment with distilled water and total dark was repeated to verify the initial results. There was no germination after 18 days. The foil was removed from containers and incubation was continued under light-dark conditions. After 13 additional days, germination was 16%.

All experiments testing the effect of temperature (see Figure 2) were conducted in total dark, except when exposed to light for a few minutes for counting purposes. Some temperature fluctuation may have occurred then also, but presumably not enough to affect results. A threshold appears to exist between 6° and 8° C; no germination was observed at the lower temperature following incubation for 30 days. An increase in temperature up to 20° C resulted in decreased germination time. Since results from 16° C and 20° C were similar, it was concluded that 16° C is a suitable temperature for conducting germination experiments.

Diluting RR Med to 1/100 (RR Med/100) appeared to have no effect on the

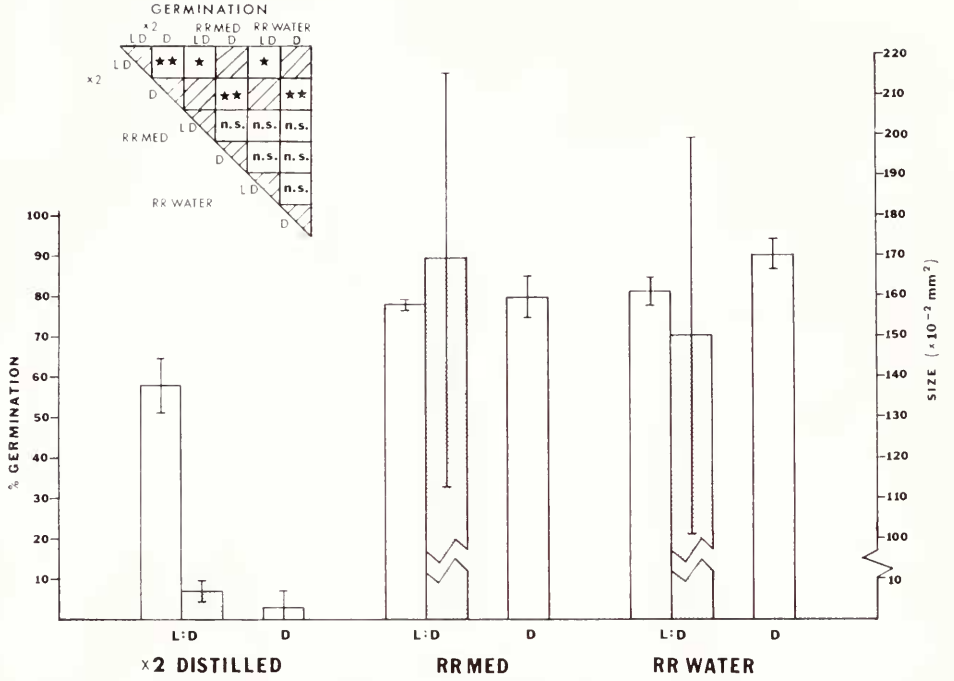

FIGURE 1. Response of gemmules of *Spongilla fragilis* to various media and light conditions. Solid bars represent per cent germination, and shaded bars represent size. Vertical lines indicate standard deviation (** = signficance at the 1% level; * = 5% level; n.s. = not significant).

rate of germination (see Figure 3). Germination decreased significantly (1% level) at RR Med/500, a value closely approximating germination in distilled water. Although RR Med 1000 supported greater germination than RR Med/500, the difference in response between the two conditions was not significant.

The effective tolerance of the organism to low concentrations of inorganic material is better illustrated by growth response. Sponges grown in RR Med 10 were similar to RR Med sponges in size and general morphology, complete with spicules (except that the number of spicules in RR Med/10 was smaller). In RR Med/100 and more dilute media, growth was significantly (1% level) retarded; the sponges were amorphous although some pores were present in RR Med/100; spicules were absent in all cases.

Clusters of spicules which remain after nitric acid digestion would seem to indicate the establishment of a skeletal network. These were noted in dilutions between RR Med and RR Med/10; however, spicule distribution was somewhat random in the dilution.

It may be concluded from this phase of the study that "normal" sponge growth in the laboratory is supported by concentrations of 0.2 mg/l Ca and Mg, 0.05 mg/l K and Na, and 0.06 mg/l SiO_2.

Exclusion of the individual compounds from RR Med did not affect rate of germination. However, growth and morphology were greatly affected (see Figures 4 and 5).

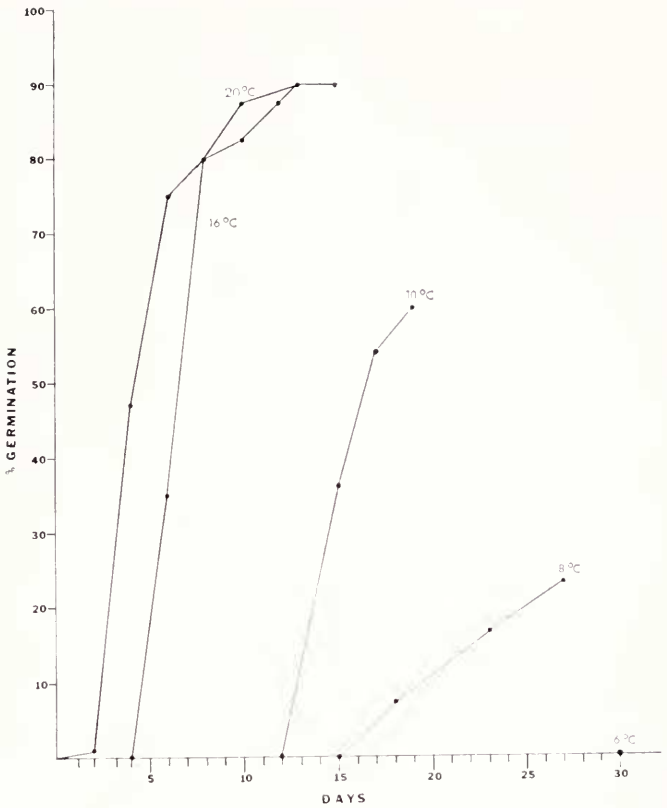

FIGURE 2. Germination response of *S. fragilis* gemmules to temperature.

RR Med lacking KCl and Na_2SiO_3 resulted in sponges of similar size; spicules were absent in the solution lacking SiO_2, but other features appeared typical. These groups differed significantly (1% level) in size from those lacking $CaCO_3$ and $MgSO_4$.

Sponges in RR Med w o $CaCO_3$ showed a tendency to be smaller than those in RR Med w/o $MgSO_4$. Morphologically these sponges were undifferentiated granular masses with no spicules; the major difference was that the cells in RR Med w/o $CaCO_3$ remained clustered around the gemmules, while those in RR Med w o $MgSO_4$ emigrated.

Omitting $CO_3^=$, Ca^{++}, $SO_4^=$, Mg^{++} in turn from RR Med produced dubious results with regard to germination. However, differences in growth were spectacular (see Figure 6). RR Med lacking either Ca or Mg produced significantly (5% level) smaller sponges than RR Med lacking SO_4 or CO_3. Although all conditions supported spicule production, only random spicules were found with Ca or Mg lacking. Negligible differentiation (although some sponges in RR Med w/o Mg had oscula) resulted although a regular skeleton was formed with both cations present. Presence of CO_3 and SO_4 (or perhaps an increased concentration of Cl) supported spicules in conjunction with either cation.

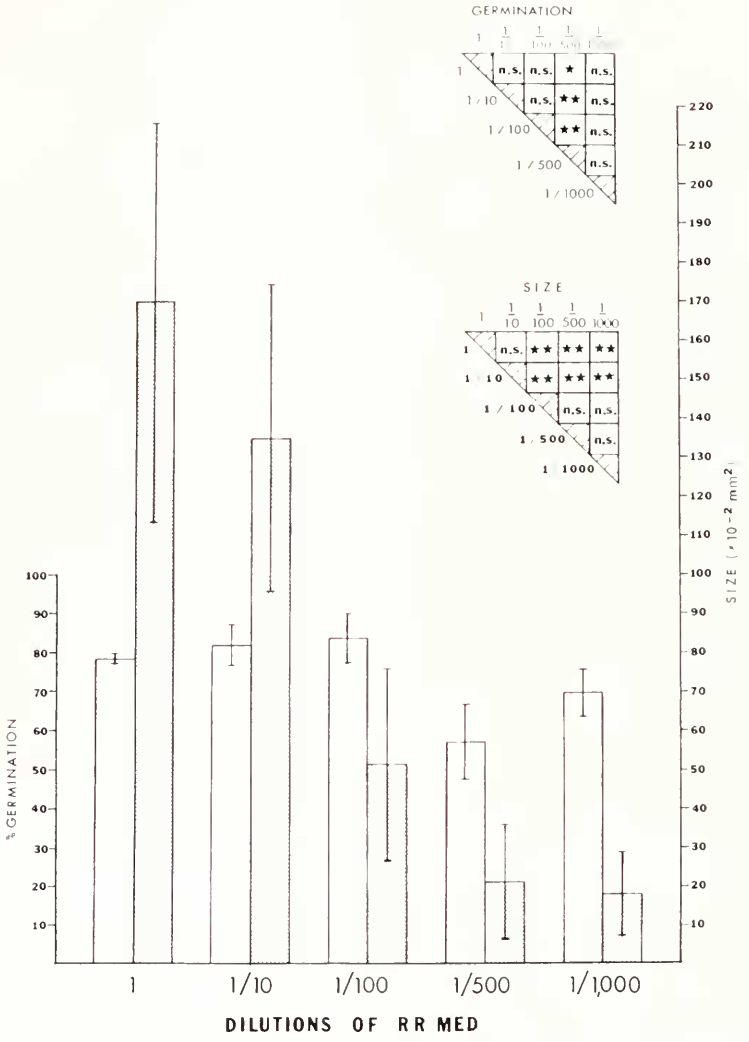

FIGURE 3. Repsonse of *S. fragilis* gemmules to dilution of basic Rapid Run Medium (RR Med); conventions as in Figure 1.

Adding the individual compounds to distilled water provided inconclusive results with respect to germination. Although $CaCO_3$ + distilled water contained significantly (1% level) larger sponges than the other compounds, they were undifferentiated and doubtfully functional.

Individual cations (as chlorides) and divalent anions (with K^+) were tested in distilled water. Only KCl resulted in a lower frequency of germination. Again Ca promoted the greatest growth of undifferentiated sponge.

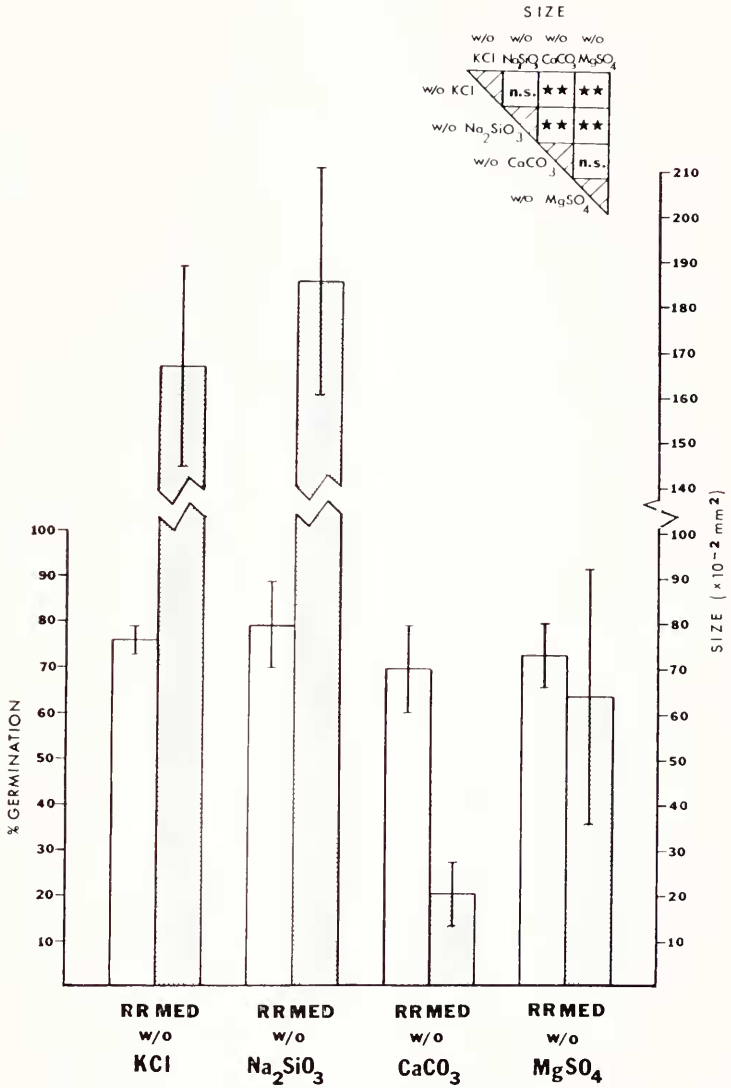

FIGURE 4. Response of *S. fragilis* gemmules to RR Med lacking each of its various components; conventions as in Figure 1.

DISCUSSION

Rasmont and Schmidt (1967) suggested that distilled water should provide a suitable medium for germination since they noted very little difference in respiration rates between gemmules of *Ephydatia fluviatilis* in it and artificial medium. It was hinted also that an increase in respiration relates to an increase in germination. Respiration was found to increase in light and decrease in dark for gemmules

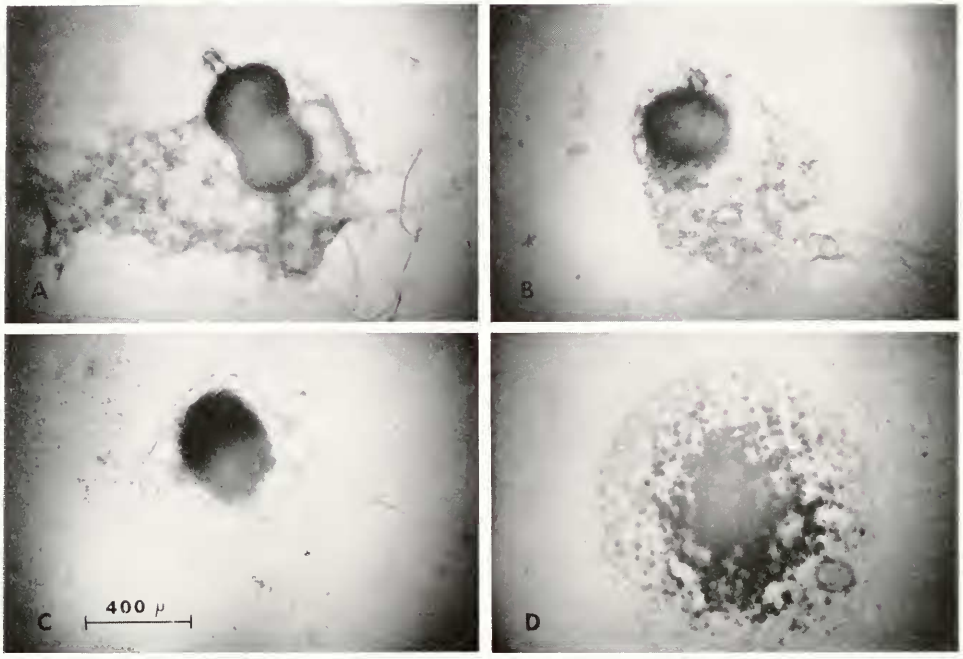

FIGURE 5. (A) Sponge germinated in RR Med lacking KCl; note spicules, osculum, and fine texture of cellular material; (B) sponge in RR Med lacking Na_2SiO_3; similar in morphology to A, but with conspicuous absence of spicules; (C) germinated gemmule in RR Med lacking $CaCO_3$; cells have barely emigrated beyond shell of gemmule; (D) cells from gemmule in RR Med lacking $MgSO_4$ have emigrated completely to form an amorphous, granular mass.

incubated in artificial medium. In addition, it was found that total light and total dark appear to be inhibitory to gemmule formation (Rasmont, 1970). The relationship of respiration to germination, however, may not be direct. Although green gemmules of *S. lacustris* respire the same in light and in dark, germination is somewhat slower in dark (Brønsted and Lovtrup, 1953).

Clearly, *S. fragilis* gemmules are not affected by light when grown in stream water or RR Med since germination and growth rates were similar in both light-dark and dark conditions. Penney (1954) stated that light does not affect the growth of freshwater sponges, but may prove limiting to symbiotic algae. Sponges in moving water tend to be located under submerged objects (Cheatum and Harris, 1953), and hence would receive very little radiation.

The effect of osmotic pressure on germination is uncertain. Rozenfeld's (1971) work on *E. fluviatilis* suggests that enzymatic action opens the micropyle and permits germination. Until that time the archaeocytes within the gemmule appear to be protected from osmotic rupture by the hydrostatic pressure of the shell. Gemmules artificially ruptured release a small percentage of archaeocytes, which may or may not differentiate depending upon the medium. It is assumed that rupture of the micropyle would also produce a small percentage of emigrant cells. If gemmules are impermeable, it is difficult to imagine why germination percentages from distilled water do not equal those from RR Med or RR water, since conditions of light, temperature, and duration of experiments were similar.

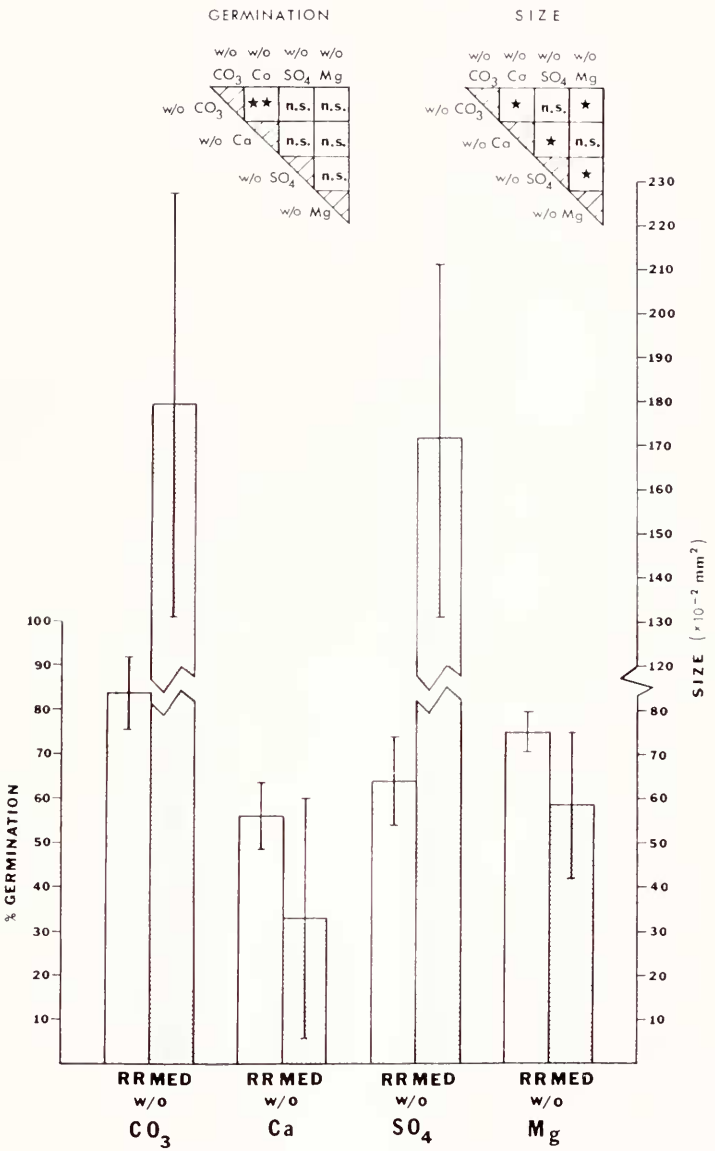

FIGURE 6. Response of *S. fragilis* gemmules to RR Med lacking individual ions; conventions as in Figure 1.

Freshwater sponges are believed to germinate between 13° and 15° C (Pennak, 1953). *S. fragilis* germinated in the laboratory at 6–8° C, and in the stream between 7.5° and 10.5° C; increasing temperature with the season would enhance the process.

Gemmulation, the formation of resistant gemmules which overwinter, began after a 5 month growing season at temperatures below 16° C; within 2 weeks thereafter the temperature had dropped back to 7–8° C. Rasmont (1968) summarized in part

that the process depends upon genetic capacity, nutrition, and relative development in *E. fluviatilis*. It was impossible in this study to test the effect of temperature on gemmulation since the sponges were not fed.

Calcium and magnesium are important controlling factors for normal cell activity because they affect cell permeability (Ruttner, 1969). Although Humphreys (1963) found calcium to be totally satisfactory in facilitating normal aggregation of marine sponge cells, it is believed (Robertson, 1941) that both divalent cations are necessary for stabilization of intercellular matrices; distilled water has a disintegrative effect on cell aggregations. Van de Vyver (1971) noted, however, that the term intercellular matrix may not be applicable to an organism lacking specific tissues, and whose cells are migrating. Amoeboid movement occurs if either cation is present, but coalescence requires both. Galtsoff (1925) suggested that each divalent ion has characteristic functions which cannot be satisfied by the other.

Jewell (1939) concluded that calcium, and not magnesium, limits sponge distribution. Freshwater sponges in Wisconsin were found in water containing as low as 0.3 mg/l Ca and 0.0 mg/l Mg; however, *S. fragilis* was limited to 2.08 and 1.0 mg/l, respectively. Macan (1961) also included calcium (among others) as a limiting factor for freshwater organisms. Excess calcium also proves to be limiting (Robertson, 1941).

Jewell (1935) has noted that silica may be limiting in strongly skeletoned forms such as *S. fragilis*. Spicule production is poor below 1.0 mg/l SiO_2, but good growth can be obtained with only a trace (Pennak, 1953). *S. fragilis* has been found in water containing as little as 0.3 mg/l SiO_2 (Wurtz, 1950).

While spicules are the first structures to be differentiated after hatching (Rasmont, 1970), spicule formation is not necessarily a suitable criterion for judging development. Sponge morphology may be influenced by environmental factors other then SiO_2; for example, high organic content may produce rapid growth without spicule formation, and no spicule pattern may be considered normal since natural conditions may induce variation (Jewell, 1935; Racek, 1969). Much differentiation and growth was achieved in RR Med w o Na_2SiO_3, but no spicules were formed.

Although calcium shows a tendency to support greater growth and differentiation than magnesium, both elements appear necessary for normal growth. RR Med/10 contains 0.2 mg/l Ca and 0.2 mg/l Mg (or 0.4 mg/l total ions) and results in a "normal" sponge; RR Med lacking either Ca or Mg (but still containing 2.0 mg/l total ions) produces a small granular mass.

Low concentrations of nutrients in a natural system may not be as limiting as expected since there would be constant replenishment due to current flow. In the preceding experiments a relatively short incubation period obviated replenishment.

The study was limited in time and scope. Since the sponges would have little or no opportunity to feed, extensive development was curtailed. Incubation was long enough to permit some growth, but short enough to prevent the cells from using up their energy reserves and disintegrating (Rasmont, 1961). Normal morphology suggests only that the organisms are functional.

The sudden appearance and disappearance of a species might suggest that one or a number of environmental factors are involved in limiting distribution. None of the important chemical parameters—calcium, alkalinity, silica, and pH (Pennak, 1953)—were limiting since there were no apparent differences among the three

sample sites on any given date. The lake appeared to have negligible influence on water chemistry.

Since chemical factors remain consistent through the course of Rapid Run, it appears that physical factors limit distribution. Under constant laboratory conditions sponges tolerate lower concentrations of inorganic material than have been recorded in field surveys. RR Med is relatively dilute, and RR water is much cleaner than many systems found to contain this species. This in itself suggests the importance of physical factors, especially in a stream. The sponges found in the quiet water around the dam are protected from current, desiccation, and suspended material which are greater in the stream above and below the dam; these sponges probably seed the stream with gemmules which may proliferate under the favorable influences found below the dam for a short distance, but only on stable substrates. No sponges were found above the lake as such a small, swift stream would not prove inviting to aquatic birds, the probable transport agent.

Summary

Gemmules of the freshwater sponge *Spongilla fragilis* were germinated and grown under a number of conditions in the laboratory using an artificial medium similar to stream water: germination was similiar under conditions of light-dark and total dark; germination was prompted by temperatures between 6–8° C; development of the young sponge was supported by ion concentrations as low as 0.2 mg/l for Mg and Ca, 0.05 mg/l for K and Na, and 0.06 mg/l for SiO_2; sponge development was inhibited by the absence of Ca and Mg, but not Na, K, or SiO_2, although no spicules were formed without SiO_2.

An annual survey was conducted to determine which physico-chemical parameters might limit the distribution of the species. As little difference with respect to chemical characteristics was noted among sampling stations and since sponges appear to tolerate low ion concentrations, it is suggested that distribution of sponges in the stream was affected by the interrelationship between current flow, suspended material, and substratum.

LITERATURE CITED

American Public Health Association, 1971. *Standard Methods for the Examination of Water and Waste Water.* [13th Edition] American Public Health Association, New York, 769 pp.

Brønsted, H. V., and E. Løvtrup, 1953. The respiration of sponge gemmules without and with symbiontic unicellular algae. *Vid. Medd. Dansk Nat. Foren.*, **115**: 145–157.

Cheatum, E. P., and J. P. Harris, Jr., 1953. Ecological observations upon the fresh-water sponges in Dallas County, Texas. *Field and Lab.*, **21**: 97–103.

Galtsoff, P. S., 1925. Regeneration after dissociation (An Experimental Study on Sponges) II. Histogenesis of *Microciona prolifera* Verra. *J. Exp. Zool.*, **42**: 223–251.

Golterman, H. L., 1969. *Methods for Chemical Analysis of Fresh Waters.* Blackwell Scientific, London, 172 pp.

Humphreys, T., 1963. Chemical dissolution on *in vitro* reconstruction of sponge cell adhesions. I. Isolation and functional demonstration of the components involved. *Develop. Biol.*, **8**: 27–47.

Hyman, L. H., 1940. *The Invertebrates (Vol. I). Protozoa through Ctenophora.* McGraw-Hill, New York and London, 726 pp.

Jewell, M. E., 1935. An ecological study of the fresh-water sponges of northeastern Wisconsin. *Ecol. Monogr.*, **5**: 461–504.

Jewell, M. E., 1939. An ecological study of the fresh-water sponges of Wisconsin, II. The influence of calcium. *Ecology*, **20**: 11–28.

Macan, T. T., 1961. Factors that limit the range of freshwater animals. *Biol. Rev.*, **36**: 151–198.

Old, M. C., 1932. Environmental selection of the fresh-water sponges (Spongillidae) of Michigan. *Trans. Amer. Microscop. Soc.*, **51**: 129–136.

Pennak, R. W., 1953. *Fresh-Water Invertebrates of the United States.* The Ronald Press Company, New York, 769 pp.

Penney, J. T., 1954. Ecological observations on the fresh-water sponges of the Savannah River Operations Area. *University of South Carolina Publishing Service III, Biology*, **1**(3): 156–172.

Racek, A. A., 1969. The freshwater sponges of Australia. *Aust. J. Mar. Freshwater Res.*, **20**(3): 267–310.

Rasmont, R., 1961. Une technique de culture des éponges d'eau douce en milieu controlé. *Ann. Soc. Roy. Zool. Belg.*, **91**: 147–156.

Rasmont, R., 1962. The physiology of gemmmulation in fresh-water sponges. *Symp. Soc. Study Develop. Growth*, **20**: 3–25.

Rasmont, R., 1963. Le rôle de la taille et de la nutrition dans le determinisme de la gemmulation chez les Spongillides. *Develop. Biol.*, **8**: 243–271.

Rasmont, R., 1968. Chemical aspects of hibernation. In M. Florkin and B. T. Scheer, Eds. *Chemical Zoology, Vol. II: Proifera, Coelenterata, and Platyhelminthes.* Academic Press, New York, 639 pp.

Rasmont, R., 1970. Some new aspects of the physiology of fresh-water sponges. *Symp. Zool. Soc. London*, **25**: 415–442.

Rasmont, R. and I. Schmidt, 1967. Mise en evidence du caractère photosensible de la respiration des gemmules de Spongillidae (Porifera). *Comp. Biochem. Physiol.*, **23**: 959–967.

Robertson, J. D., 1941. The function and metabolism of calcium in the invertebrata. *Biol. Rev.*, **16**: 106–133.

Rozenfeld, R., 1970. Inhibition du developpement des gemmules de Spongillides: Specificité et moment d'action de la gemmulostasine. *Arch. Biol.*, **81**: 193–214.

Rozenfeld, R., 1971. Effets de la perforation de la coque des gemmules d'*E. fluviatilis* (Spongillides) sur leur developpement ulterieut en présence de gemmulostasine. *Arch. Biol.*, **82**(2): 103–113.

Ruttner, F., 1969. *Fundamentals of Limnology.* University of Toronto Press, Toronto, 295 pp.

Van de Vyver, G., 1971. Mise en evidence d'un facteur d'agregation chez l'eponge d'eau douce. *E. fluviatilis. Ann. Embryol. Morphogr.*, **4**(4): 373–381.

Wurtz, C. B., 1950. Fresh-water sponges of Pennsylvania and adjacent states. *Notulae Naturae*, **228**: 1–10.